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Heat Stability of Strawberry Anthocyanins in Model Solutions Containing Natural Copigments Extracted from Rose (*Rosa damascena* Mill.) Petals

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Thermal degradation and color changes of purified strawberry anthocyanins in model solutions were studied upon heating at 85 °C by HPLC-DAD analyses and CIELCh measurements, respectively. The anthocyanin half-life values increased significantly due to the addition of rose (*Rosa damascena* Mill.) petal extracts enriched in natural copigments. Correspondingly, the color stability increased as the total color difference values were smaller for anthocyanins upon copigment addition, especially after extended heating. Furthermore, the stabilizing effect of rose petal polyphenols was compared with that of well-known copigments such as isolated kaempferol, quercetin, and sinapic acid. The purified rose petal extract was found to be a most effective anthocyanin-stabilizing agent at a molar pigment/copigment ratio of 1:2. The results obtained demonstrate that the addition of rose petal polyphenols slows the thermal degradation of strawberry anthocyanins, thus resulting in improved color retention without affecting the gustatory quality of the product.

KEYWORDS: Strawberry anthocyanins; thermal degradation; color stability; copigmentation; *Rosa damascena* Mill.; polyphenols

INTRODUCTION

Anthocyanins not only are important in determining the esthetic value of foods and beverages but also play a significant role from a nutritional point of view, being bioavailable dietary antioxidants (*1*). The attractive bright red color is the major visual quality attribute strongly affecting consumer acceptance of both fresh and processed strawberry fruits. Unfortunately, accelerated pigment degradation and color loss occur during conventional processing and storage (*2*) due to the low total content of strawberry anthocyanins (*3*) and their inherent heat and light sensitivity (*4*). For this reason, synthetic colorants such as ponceau 4R (E 124) have commonly been used for improving the visual appearance of strawberry products. However, the safety of synthetic colorants is questioned, because numerous examples in the past gave evidence of serious health issues when these synthetic additives (e.g., Sudan dyes) were used. A very recent study has also revealed significant side effects following consumption of drinks with added artificial food colorants in 3- and 8/9-year-old children with regard to the degree of attention-deficit hyperactivity disorder (*5*). Therefore, the substitution of synthetic food colorants by their natural counterparts and the development of methods to stabilize natural pigments are of utmost importance to meet consumer demands for safer and healthier food.

Intermolecular copigmentation is a natural color-stabilizing mechanism in which anthocyanins and copigments, mostly polyphenols, form complexes by weak hydrophobic and $\pi-\pi$ interactions, thus explaining the hyperchromic and bathochromic effects. Copigmentation is assumed to be responsible for the color variations that occur in flowers in a pH range at which isolated anthocyanins are virtually colorless (*6*). The significance of this phenomenon, for example, for color evolution during red wine aging has been thoroughly investigated (*7*). With respect to strawberry anthocyanins, an increase in color intensity has been observed after the addition of phenolic acids acting as copigments (*8, 9*). However, due to the high pigment/copigment ratios required for color stabilization, the flavor threshold concentrations may be exceeded, promoting an astringency perception caused by the phenolic acids (*10*). Moreover, phenolic acids are susceptible to oxidation with subsequent browning, thus negatively affecting the visual appearance of processed foods (*11*).

Recently, industrially distilled petals of *Rosa damascena* Mill. were established as a rich source of polyphenols, particularly

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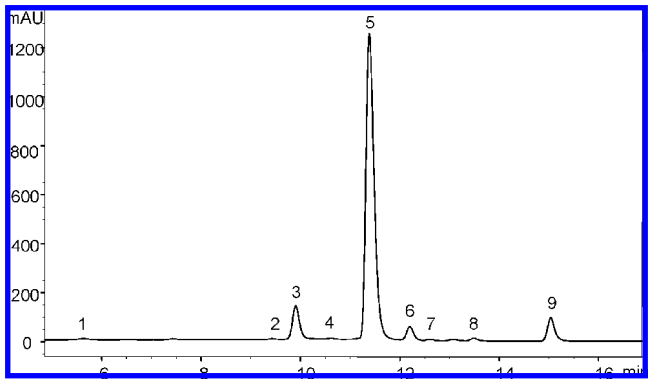

Figure 1. HPLC separation (520 nm) of anthocyanins from purified strawberry extract. For peak assignment see **Table 1**.

Table 1. LC-MS Data of Anthocyanins from Purified Strawberry Extract

peak	retention time (min)	$[M]^+$ (*m*/*z*)	MS/MS fragments (*m*/*z*)	peak assignment
1	5.6	595	433/271	pelargonidin diglycoside
2	9.1	433	271	pelargonidin hexoside
3	9.9	449	287	cyanidin 3-*O*-glucoside[a]
4	10.6	433	271	pelargonidin hexoside
5	11.4	433	271	pelargonidin 3-*O*-glucoside[a]
6	12.2	579	271	pelargonidin 3-*O*-rutinoside
7	12.6	433	271	pelargonidin hexoside
8	13.5	535	287	cyanidin 3-*O*-malonylglucoside
9	15.0	519	271	pelargonidin 3-*O*-malonylglucoside

[a] Identification with a standard.

flavonols (*12*), which were shown to be highly effective natural copigments at low pigment/copigment ratios (*13*).

Therefore, the present study aimed at the evaluation of the heat stability of purified strawberry anthocyanins in model solutions with added rose petal extracts enriched in natural copigments. Additionally, the stabilizing effect of rose petal polyphenols was compared with that of other well-known copigments such as isolated flavonols and phenolic acids.

MATERIALS AND METHODS

Chemicals. Cyanidin 3-*O*-glucoside and pelargonidin 3-*O*-glucoside were from Polyphenols (Sandnes, Norway). Quercetin (Q), Q 3-*O*-glucoside, Q 3-*O*-galactoside, Q 3-*O*-rhamnoside, Q 3-*O*-xyloside, Q 3-*O*-rutinoside, kaempferol, and kaempferol 3-*O*-glucoside were obtained from Roth (Karlsruhe, Germany). All other reagents and solvents were purchased from VWR (Darmstadt, Germany) and were of analytical or HPLC grade. The adsorbent resin Amberlite XAD 16 HP was provided by Rohm & Haas (Darmstadt, Germany). Deionized water was used throughout.

Extraction and Purification of Anthocyanins and Phenolic Copigments. Frozen strawberries (*Fragaria* × *ananassa* Duch., cv. Senga Sengana, harvest 2007) were thawed and manually squeezed in a beaker. The homogenized purée (approximately 2 kg) was extracted overnight at 4 °C using 2.5 L/kg of methanol acidified with hydrochloric acid (1%, v/v). The extraction mixture was filtered through a paper filter (Macherey-Nagel, Düren, Germany) on a Büchner funnel, and the organic solvent was evaporated under vacuum (30 °C). To remove sugars, salts, and amino acids from the crude extracts, samples were purified using a column (465 × 30 mm i.d.) filled with Amberlite XAD 16 HP. Prior to sample application, the resin was conditioned and equilibrated by rinsing with 500 mL of methanol and 1000 mL of water, acidified with trifluoroacetic acid (TFA, pH 2). Subsequently, 250 mL of the aqueous strawberry extract was applied and the column rinsed with 1000 mL of acidified water (pH 2). For elution of the pigments at least 500 mL of a mixture of methanol and acidified water (TFA, pH 2) (95:5, v/v) was applied until the column was colorless. The organic solvent of the eluate was evaporated under vacuum (30 °C). To separate anthocyanins from colorless phenolics, further purification was performed by extracting the aqueous phase three times with the same volume of ethyl acetate. After evaporation and concentration under vacuum (30 °C), the residue was lyophilized for 72 h. Prior to HPLC analysis, 10 mg of the lyophilized extract was dissolved in 25 mL of deionized water, and the solution was membrane-filtered (0.45 μm).

The byproduct originating from water−steam distillation of *R. damascena* Mill. petals was supplied by Nara Geo Distillery (Plovdiv, Bulgaria). After pressing of the wet material, the pomace obtained was hot air-dried (60 °C, 6 h). Rose petal polyphenols were extracted with 30% aqueous ethanol using approximately 400 g of finely milled pomace (particle size < 4 mm) at a liquid to solid ratio of 20:1 (v/w). After 1 h of stirring at ambient temperature, the extraction mixture was filtered using a paper filter, and the organic solvent was evaporated under vacuum (30 °C). The extract obtained was either lyophilized for 72 h (crude extract) or further purified on a column (465 × 30 mm i.d.) filled with XAD 16 HP. Prior to sample application, the resin was conditioned and equilibrated as described above. Then 250 mL of the crude extract was applied and the column subsequently rinsed with 1000 mL of acidified water (TFA, pH 2). For the elution of the rose petal phenolics 500 mL of a mixture of methanol and acidified water (pH 2) (95:5, v/v) was applied to the column. After evaporation and concentration under vacuum (30 °C), the polyphenols were lyophilized for 72 h. For HPLC analysis 50 mg of the lyophilized sample was dissolved in 25 mL of deionized water, and the solution was membrane-filtered (0.45 μm).

Preparation of Model Solutions. Stock solutions of strawberry and rose petal extracts were prepared on the basis of total anthocyanin and total phenolic contents, respectively, as determined by HPLC. For this purpose, individual anthocyanins were quantified as pelargonidin 3-*O*-glucoside equivalents, and individual polyphenols in the rose petal extract were quantified as kaempferol 3-*O*-glucoside equivalents, respectively. The stock solutions were prepared in McIlvaine buffer (0.1 M, pH 3.4). Model solutions of strawberry anthocyanins (1×10^{-4} M), with and without copigment addition, were obtained by mixing appropriate volumes of both stock solutions and were then left for equilibration (30 min at 25 °C).

Heat Stability Tests. Heating experiments were performed in a water bath at 85 °C in Pyrex tubes (100 × 14 mm i.d., Bibby Sterilin, Stone Staffs, U.K.) equipped with a magnetic stirrer and plastic screw caps. After 30, 60, 90, and 120 min, respectively, the tubes were immediately cooled in an ice bath to stop thermal degradation of phenolic compounds. An aliquot of each sample was passed through a PTFE filter (0.45 μm) and analyzed by HPLC-DAD to quantify individual anthocyanins. The remainder was used for colorimetric measurements. An unheated sample was used as a control and analyzed in the same way.

HPLC-DAD and LC-MS Analyses. The identification and quantification of strawberry anthocyanins and rose petal polyphenols were performed using an Agilent HPLC, series 1100 (Agilent, Waldbronn, Germany), equipped with ChemStation software, a model G1322A degasser, a model G1312A binary gradient pump, a model G1329/G1330A thermoautosampler, a model G1316A column oven, and a model G1315A diode array detector. The column used was a Phenomenex (Torrance, CA) Synergi Hydro-RP 80A (150 × 3.0 mm i.d., 4 μm particle size), with a C18 guard column (4.0 mm × 3.0 mm i.d.), operated at 25 °C. The diode array detector was set at an acquisition range of 200−600 nm at a spectral acquisition rate of 1.25 scans/s (peak width = 0.2 min).

For the determination of strawberry anthocyanins a chromatographic system developed for anthocyanin analysis in red wine was applied (*14*). The mobile phase consisted of water/formic acid/acetonitrile (87:10:3, v/v/v; eluent A) and of water/formic acid/acetonitrile (40:10:50, v/v/v; eluent B). The gradient program was as follows: 6−30% B (15 min), 30−50% B (15 min), 50−60% B (5 min), 60−100% B (5 min), 100−6% B (5 min), 6% B isocratic (5 min). Monitoring was performed at 520 nm at a flow rate of 0.4 mL/min. The injection volume was 10 μL. Pelargonidin- and cyanidin-based anthocyanins were quantified using a calibration curve of pelargonidin 3-*O*-glucoside and cyanidin 3-*O*-glucoside, respectively, including a molecular weight correction factor (*15*). Total anthocyanin contents were calculated as the sum of

Table 2. LC-MS Data and Contents of Phenolic Compounds in Crude and Purified Rose Petal Extracts

peak	retention time(min)	$[M - H]^-$ (*m*/*z*)	MS/MS fragments (*m*/*z*)	peak assignment	content (mg/100 g dwb[a]) crude extract	content (mg/100 g dwb[a]) purified extract
1	27.6	615	463/301	quercetin galloylhexoside	62 ± 2	195 ± 6
2	28.2	615	463/301	quercetin galloylhexoside	35 ± 1	96 ± 2
3	29.9	609	301	quercetin 3-*O*-rutinoside[b]	295 ± 10	556 ± 15
4	30.4	463	301	quercetin 3-*O*-galactoside[b]	630 ± 13	1336 ± 36
5	31.1	463	301	quercetin 3-*O*-glucoside[b]	562 ± 6	1255 ± 41
6a	32.8	615	301	quercetin galloylhexoside	122 ± 4	325 ± 9
6b	32.8	433	301	quercetin 3-*O*-xyloside[b]	tr[c]	tr
7	33.9	447	285	kaempferol hexoside	459 ± 11	1038 ± 34
8	34.2	593	285	kaempferol disaccharide	100 ± 2	254 ± 8
9	34.6	609	301	quercetin disaccharide	286 ± 3	642 ± 22
10a	35.5	447	301	quercetin 3-*O*-rhamnoside[b]	tr	tr
10b	35.5	447	285	kaempferol 3-*O*-glucoside[b]	1647 ± 33	3900 ± 106
11	37.5	599	285	kaempferol galloylhexoside	112 ± 1	284 ± 5
12	38.2	417	285	kaempferol pentoside	147 ± 3	322 ± 7
13	39.4	593	285	kaempferol disaccharide	102 ± 3	186 ± 6
14	39.6	417	285	kaempferol pentoside	276 ± 5	638 ± 13
15	40.8	431	285	kaempferol deoxyhexoside	344 ± 7	810 ± 20
16	41.2	651	609/301	quercetin acetyldisaccharide	73 ± 2	153 ± 4
17	45.0	609	463/301	quercetin disaccharide	20 ± 0	40 ± 1
18	47.0	635	285	kaempferol acetyldisaccharide	115 ± 1	230 ± 8
19	47.7	301		quercetin[b]	4 ± 0	14 ± 0
20	49.9	593	285	kaempferol disaccharide	527 ± 18	866 ± 28
21	51.2	593	285	kaempferol disaccharide	34 ± 1	64 ± 1
22	56.7	285	257	kaempferol[b]	16 ± 0	36 ± 1
				total	5968	13240

[a] Dry weight base. [b] Identification with a standard. [c] Trace.

Table 3. Total Anthocyanin Contents in Model Solutions with Added Rose Petal Polyphenolics (Purified Extract) and Half-Life Values of Strawberry Anthocyanins upon Heating at 85 °C

heating time (min)	molar pigment/copigment ratio 1:0 (control)	1:1	1:2	1:3	1:4	1:5
	Anthocyanin Content (Milligrams per Liter)					
0	40.5 ± 0.1	40.0 ± 0.1	39.8 ± 0.1	40.0 ± 0.3	39.4 ± 0.1	39.6 ± 0.1
30	35.9 ± 0.1	36.5 ± 0.2	36.2 ± 0.3	36.3 ± 0.2	35.8 ± 0.1	35.0 ± 0.1
60	32.3 ± 0.1	33.6 ± 0.3	34.0 ± 0.0	33.5 ± 0.8	32.7 ± 0.1	32.4 ± 0.1
90	28.9 ± 0.2	30.7 ± 0.3	30.3 ± 0.2	30.0 ± 0.3	29.4 ± 0.2	28.7 ± 0.2
120	25.7 ± 0.2	27.6 ± 0.4	27.7 ± 0.3	27.1 ± 0.1	26.9 ± 0.2	26.8 ± 0.5
	Half-Life (Minutes)					
$t_{1/2}$ (R)[a]	182.4 (0.99)	223.6 (0.99)	231.0 (0.99)	216.6 (0.99)	216.6 (0.99)	210.0 (0.99)

[a] R = correlation coefficient.

individually quantified compounds. Thermal degradation kinetics of total anthocyanins was determined by calculation of the half-life values as described by Sadilova et al. (*16*).

For the determination of rose petal polyphenols the mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and of 0.5% acetic acid in water and acetonitrile (50:50, v/v; eluent B). The gradient program was as follows: 10−60% B (50 min), 60−100% B (10 min), 100−10% B (5 min), 10% B isocratic (5 min). Monitoring was performed at 370 nm at a flow rate of 0.4 mL/min. The injection volume of all samples was 8 μL. Contents of individual compounds were determined using calibration curves of quercetin, quercetin 3-*O*-rutinoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-xyloside, kaempferol, and kaempferol 3-*O*-glucoside, including a molecular weight correction factor (*15*). Total polyphenolic contents were calculated as the sum of individually quantified compounds.

The HPLC system described above was connected in series with a Bruker (Bremen, Germany) Esquire 3000+ ion trap mass spectrometer fitted with an electrospray ionization (ESI) source. Data acquisition and processing were performed using Esquire control software. Positive ion mass spectra, in the range of *m*/*z* 50−1500, and negative ion mass spectra, in the range of *m*/*z* 50−2800, were recorded for analysis of anthocyanins and colorless polyphenols, respectively. Nitrogen was used as the dry gas at a flow rate of 9.0 L/min and at a pressure of 45.0 psi. The nebulizer temperature was set at 365 °C. Collision-induced dissociation (CID) was performed with a fragmentation amplitude of 1.0 V using helium as collision gas at a pressure of 1.17×10^{-8} bar.

Color Measurements. All samples were analyzed with a UV−vis spectrophotometer (Perkin-Elmer, Überlingen, Germany) equipped with UV−vis (UV-Winlab version 2.85.04) and color (Wincol version 2.05) software (Perkin-Elmer, Norwalk, CT) using 1 cm path length cuvettes. For the color measurements, the unheated samples were diluted with McIlvaine buffer (pH 3.4) to reach maximum absorption values of 0.80 ± 0.02, and the same dilutions were subsequently applied after heating. After 30 min of equilibration at 25 °C, visible spectra were recorded in a range of 380 to 780 nm. CIELCh color coordinates were determined using illuminant D65 and 10° observer angle. The total color difference of heated samples as compared to the respective untreated solutions was calculated according to the method of Gonnet (*17*).

Statistical Analysis. The results reported in the present study are the mean values of at least two determinations, and the coefficients of variation, expressed as the percentage ratio between the standard deviations and the mean values, were found to be below 3% in all cases. Linear regression analysis was performed using the statistical package of Microsoft Excel.

Table 4. CIELCh Color Coordinates and Total Color Difference Values of Strawberry Anthocyanins upon Heating at 85 °C in Model Solutions with Added Rose Petal Polyphenolics (Purified Extract)

heating time (min)	molar pigment/copigment ratio					
	1:0 (control)	1:1	1:2	1:3	1:4	1:5
			Lightness (L^*)			
0	76.1 ± 0.2	74.8 ± 0.1	74.3 ± 0.4	71.6 ± 0.3	69.3 ± 0.1	68.8 ± 0.1
30	78.5 ± 0.4	76.2 ± 0.9	75.3 ± 0.8	74.6 ± 0.3	72.1 ± 0.2	72.5 ± 0.3
60	78.7 ± 0.8	76.3 ± 0.7	75.4 ± 0.5	74.4 ± 0.3	71.0 ± 0.5	71.9 ± 0.1
90	79.0 ± 0.1	75.9 ± 0.4	75.3 ± 0.2	74.0 ± 0.1	70.6 ± 0.5	71.5 ± 0.4
120	79.7 ± 0.2	77.0 ± 0.6	74.3 ± 0.1	74.9 ± 0.2	70.6 ± 0.8	70.7 ± 0.6
			Chroma (C^*)			
0	41.3 ± 0.1	41.4 ± 0.1	41.1 ± 0.2	39.9 ± 0.6	39.6 ± 0.8	37.3 ± 0.4
30	40.1 ± 0.1	40.3 ± 0.9	39.5 ± 0.2	39.1 ± 0.3	38.5 ± 0.6	36.4 ± 0.4
60	38.2 ± 0.5	39.1 ± 0.7	38.6 ± 0.7	37.5 ± 0.4	37.1 ± 1.0	35.7 ± 0.1
90	35.4 ± 0.2	37.1 ± 0.4	37.4 ± 0.3	36.5 ± 0.1	36.4 ± 0.8	34.5 ± 0.1
120	34.4 ± 0.2	36.9 ± 0.5	36.4 ± 0.3	36.2 ± 0.3	35.7 ± 0.9	33.7 ± 0.2
			Hue Angle (h°)			
0	41.3 ± 0.1	36.3 ± 0.2	35.5 ± 0.3	35.6 ± 0.7	37.3 ± 0.2	39.2 ± 0.1
30	40.2 ± 0.4	35.6 ± 0.5	34.7 ± 0.4	35.3 ± 0.1	35.9 ± 0.3	39.0 ± 0.2
60	40.0 ± 0.2	35.8 ± 0.1	35.4 ± 0.3	35.6 ± 0.4	38.9 ± 0.1	41.0 ± 0.2
90	40.2 ± 0.5	36.4 ± 0.2	36.1 ± 0.2	37.0 ± 0.1	40.4 ± 0.2	42.2 ± 0.3
120	40.4 ± 0.1	36.7 ± 0.1	36.9 ± 0.2	37.7 ± 0.3	41.2 ± 0.3	43.6 ± 0.2
			Color Difference (ΔE^*)			
30	2.9	1.9	2.0	3.1	3.3	3.7
60	4.3	2.7	2.8	3.7	3.5	3.9
90	4.8	4.4	3.9	4.4	4.6	4.9
120	7.9	5.0	4.9	5.4	5.7	6.0

RESULTS AND DISCUSSION

Characterization and Quantification of Strawberry Anthocyanins. The anthocyanin profile of the purified strawberry extract (**Figure 1**; **Table 1**) was in accordance with previous studies (*18*−*20*). Pelargonidin 3-glucoside (**5**) was the predominant compound (80.6%) followed by cyanidin 3-glucoside (**3**; 8.1%), pelargonidin 3-malonylglucoside (**9**; 6.7%) and pelargonidin 3-rutinoside (**6**; 5.4%). Some minor compounds (**1**, **2**, **4**, **7**, and **8**) having a total peak area of <2% were present only in trace amounts. The total anthocyanin content of the purified strawberry extract was 14.8 g/100 g on a dry matter base.

Characterization and Quantification of Rose Petal Polyphenols. The identification and quantification of colorless phenolic compounds in the rose petal extracts are illustrated in **Table 2**. Consistent with previous findings (*12, 21, 22*), among the 24 major compounds analyzed were 11 kaempferol and 11 quercetin glycosides, along with their aglycones. The kaempferol glycosides accounted for 65% of the total compounds that were quantified for both the crude and purified extract, with kaempferol 3-*O*-glucoside being the predominant compound (∼30%).

Amberlite XAD 16 HP was used in this study for the adsorptive purification of rose petal polyphenolics because it has been approved for food use by the U.S. Food and Drug Administration (FDA). Furthermore, it has been successfully applied to the recovery of phenolic compounds from apple (*23*) and grape pomace (*24*) extracts. After adsorptive purification, the total flavonol concentration was 2.2-fold increased compared to the crude extract applied to the adsorbent column. However, as reported for the adsorptive recovery of apple polyphenols (*25*), HPLC analyses revealed different affinities of individual compounds, thus enabling the selective enrichment of certain polyphenols. Interestingly, the contents of galloylated glycosides were 2.5−3.2 times higher in the purified extract, whereas the average degree of enrichment for the nongalloylated compounds was 2.1 (**Table 2**). This might be of particular importance because the presence of galloyl moieties in flavan-3-ol structures has been demonstrated to boost the copigmentation effect (*26*). For this reason, the purified rose petal extract was used in the subsequent experiments.

Heat Stability of Copigmented Strawberry Anthocyanins. To exclude the possible influence of copigmentation on the total anthocyanin content determination, which is usually performed using the pH differential spectrophotometric method, pigment degradation was assessed by HPLC analyses in the present study. As shown in **Table 3**, with increased heating times pigment contents were significantly lowered. After 2 h, relative pigment concentrations amounting to 63 and 70% were observed for non-copigmented and copigmented (molar ratio 1:2) strawberry anthocyanins, respectively, revealing an enhanced stability in the presence of colorless rose petal polyphenols. As indicated by the high values of the correlation coefficient (R), anthocyanin degradation ideally followed first-order reaction kinetics (**Table 3**). These findings are in accordance with previous studies reporting first-order degradation kinetics for anthocyanins both in model systems (*27*−*29*) and in real food matrices (*30*−*32*). The anthocyanin half-life values ($t_{1/2}$) increased significantly due to the addition of purified rose petal extract, reflecting lower pigment degradation rates in the presence of phenolic copigments, which is in agreement with the results of Brenes et al. (*33*). Interestingly, maximum heat stability was reached at a low pigment/copigment ratio (1:2), whereas higher concentrations of copigments resulted in a moderate decline of pigment stability. This fact is of particular importance from a sensory point of view, implying that the most effective supplementation of rose petal extracts to strawberry products would not affect the typical flavor of these products.

Color changes of copigmented strawberry anthocyanins as a function of heating time were monitored by CIELCh measurements (**Table 4**). The solutions were adjusted to identical tinctorial strengths, which allows direct comparison of lightness (L^*), chroma (C^*), and hue angle (h°) values. Added rose petal polyphenols caused concentration-dependent decreases of L^* values, confirming the effects of copigmentation as assessed by the colorimetric approach (*34*). The hue angle values changed significantly upon copigment addition, reflecting a more reddish color tonality, especially at low pigment/copigment ratios. In general, decreasing pigment concentrations during thermal treatment were associated with declining C^* values, indicating color purity loss. However, the relative changes of C^* values were smaller than the corresponding decrease in anthocyanin concentrations, which implies that copigmentation effects may mask the pigment degradation that occurred as a result of heating. Moreover, the color stability increased due to the addition of colorless rose petal polyphenols as the total color difference values ΔE^* were smaller for the copigmented anthocyanins, especially upon extended heating. In accordance with the results obtained by HPLC analyses, the color stability was higher at a low pigment/copigment ratio (1:2).

In a second series of experiments the stabilizing effect of rose petal polyphenols was compared with that of well-known copigments such as isolated flavonols and phenolic acids (**Tables 5** and **6**). Rose petal polyphenols were found to be the most effective anthocyanin-stabilizing agents, significantly exceeding the effects even of kaempferol, although kaempferol glycosides dominated the phenolic profile of the purified rose petal extract. This may be attributed to the glycosylation pattern of the rose petal polyphenols, some of them being acylated, because both the rose petal phenolics and the isolated polyphenols were applied at equimolar concentrations (2×10^{-4} M). Moreover, the existence of synergistic effects in the case of rose petal

Table 5. Total Anthocyanin Contents in Model Solutions with Added Copigments (Molar Ratio 1:2) and Half-Life Values of Strawberry Anthocyanins upon Heating at 85 °C

heating time (min)	rose petal polyphenolics		kaempferol	quercetin	sinapic acid
	crude extract	purified extract			
	Anthocyanin Content (Milligrams per Liter)				
0	37.5 ± 1.1	39.8 ± 0.1	39.5 ± 0.1	40.2 ± 0.4	40.2 ± 0.1
30	33.2 ± 0.5	36.2 ± 0.0	36.0 ± 0.4	35.8 ± 0.2	35.7 ± 0.4
60	29.3 ± 0.2	34.0 ± 0.0	32.1 ± 0.1	32.9 ± 0.3	33.1 ± 0.3
90	26.3 ± 0.4	30.3 ± 0.2	29.8 ± 0.2	29.8 ± 0.5	29.5 ± 0.3
120	24.1 ± 0.3	27.7 ± 0.3	26.7 ± 0.1	26.7 ± 0.0	26.2 ± 0.3
	Half-Life (Minutes)				
$t_{1/2}$ (R)[a]	187.3 (0.99)	231.0 (0.99)	216.6 (0.99)	210.0 (0.99)	198.0 (0.99)

[a] R = correlation coefficient.

Table 6. CIELCh Color Coordinates and Total Color Difference Values of Strawberry Anthocyanins upon Heating at 85 °C in Model Solutions with Added Copigments (Molar Ratio 1:2)

heating time (min)	rose petal polyphenolics		kaempferol	quercetin	sinapic acid
	crude extract	purified extract			
	Lightness (L^*)				
0	68.6 ± 0.3	74.3 ± 0.4	77.1 ± 0.3	75.3 ± 0.2	77.4 ± 0.2
30	70.9 ± 0.1	75.3 ± 0.8	78.2 ± 0.4	78.6 ± 0.1	78.6 ± 0.2
60	71.4 ± 0.2	75.4 ± 0.5	79.0 ± 0.1	79.7 ± 0.3	79.1 ± 0.3
90	71.3 ± 0.6	75.3 ± 0.2	78.2 ± 0.1	78.6 ± 0.8	78.3 ± 0.4
120	71.1 ± 0.1	74.3 ± 0.1	79.6 ± 0.2	79.3 ± 0.1	80.2 ± 0.4
	Chroma (C^*)				
0	40.4 ± 0.2	41.1 ± 0.2	41.2 ± 0.3	38.8 ± 0.2	42.3 ± 0.1
30	38.8 ± 0.4	39.5 ± 0.2	38.7 ± 0.1	37.7 ± 0.2	39.6 ± 0.2
60	36.8 ± 0.5	38.6 ± 0.7	37.4 ± 0.2	36.7 ± 0.1	38.5 ± 0.3
90	35.4 ± 0.3	37.4 ± 0.3	35.7 ± 0.3	34.5 ± 0.3	36.3 ± 0.4
120	34.2 ± 0.4	36.4 ± 0.3	35.0 ± 0.2	33.2 ± 0.1	35.4 ± 0.2
	Hue Angle ($h°$)				
0	45.3 ± 0.1	35.5 ± 0.3	41.6 ± 0.1	41.0 ± 0.1	40.1 ± 0.1
30	44.1 ± 0.3	34.7 ± 0.4	40.3 ± 0.3	38.9 ± 0.1	39.3 ± 0.5
60	44.2 ± 0.0	35.4 ± 0.3	40.0 ± 0.2	38.9 ± 0.3	39.1 ± 0.2
90	43.8 ± 0.3	36.1 ± 0.2	40.4 ± 0.1	39.4 ± 0.4	39.3 ± 0.1
120	43.4 ± 0.1	36.9 ± 0.2	39.3 ± 0.2	39.7 ± 0.1	39.2 ± 0.2
	Color Difference (ΔE^*)				
30	3.0	2.0	3.0	4.1	3.1
60	4.6	2.8	4.5	5.3	4.3
90	5.8	3.9	5.8	5.6	6.1
120	6.9	4.9	7.1	7.0	7.5

polyphenols may also be assumed. Purification considerably improved the efficiency of the rose petal extract. Because differences in the HPLC profile of rose petal polyphenolics upon heating were not observed (data not shown), it is assumed that through adsorptive isolation nonphenolic compounds capable of accelerating anthocyanin degradation are removed. In contrast to previous findings (*8, 9*), sinapic acid did not have a significant anthocyanin-stabilizing effect, which is probably due to the low pigment/copigment ratio used in the present study. In general, these findings are in accordance with the results obtained by the colorimetric measurements. Interestingly, despite differences in the pigment degradation rate (**Table 5**), similar ΔE^* values (**Table 6**), but higher than that for the purified rose petal extract, were observed for the other copigments used. These results support the assumption that the copigmentation effects could, to a certain extent, mask the pigment degradation that took place during heating.

In conclusion, the results presented here demonstrate that the addition of colorless rose petal polyphenols acting as copigments significantly reduces the thermal degradation of strawberry anthocyanins in model solutions, thus bringing about enhanced color stability, even at a molar pigment/copigment ratio of 1:2. Whereas the application of a new firming process, which has recently been developed, significantly improves strawberry texture (*35*), color retention, particularly after heat exposure, remains a technological challenge. Therefore, our current activities aim at the color stabilization of texture-improved canned strawberries by adding purified rose petal extracts instead of ponceau 4R (E 124). In contrast to the addition of rosemary and thyme extracts, which have been used as anthocyanin-stabilizing agents at a 1:100 copigmentation ratio (*36*), rose petal polyphenols are effective at much lower concentrations, thus allowing an economically viable stabilization method. However, a deeper insight needs to be gained into the interactions of both pigments and copigments with matrix compounds such as cell wall material, which may affect the efficiency of rose petal polyphenols in a complex food system.

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